GUP1 of _Saccharomyces cerevisiae_ Encodes an _O_-Acyltransferase Involved in Remodeling of the GPI Anchor

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Supplemental Material

This article contains the following supporting material:

- Figure 1 - ***Yor175c*· strain remodels GPI anchors normally.**
  Cells of indicated genotype were labeled at 30°C with [^{3}H]inositol, lipids of the GPI anchors were extracted and analyzed by TLC/fluorography using solvent 2. 5· represents *are1*· *are2*· *gup1*· *gup2*· *yor175c*·. The first lane contains the [^{3}H]inositol labeled free lipids of wt cells (wt, FL).

- Figure 2 - ***gup1*p does not act on free GPI lipids.**
  A, Microsomes were prepared from the indicated strains and incubated with UDP-[^{3}H]GlcNAc, GDP-Man, nikkomycin, tunicamycin, CoA and ATP at 30°C for 90 min as described (Imhof et al., 2004), either in the absence (lanes 1, 3, 5, 7), or the presence (lanes 2, 4, 6, 8) of 0.1mM C26:0-CoA (ANAWA trading SA, Switzerland). Lipids were extracted, desalted, and analyzed by TLC/fluorography. **B,** the same microsomes were labeled as above in the absence of C26:0-CoA, were then washed 2 times in Hepes-KOH buffer pH 7.4 (HKLM buffer (Morita et al., 2000)) by centrifugation (15,000 g, 30', 4°C), and were either directly subject to lipid extraction (lanes 1, 3, 5, 7) or further incubated with 0.1 mM C26:0-CoA for 30 °C (lanes 2, 4, 6, 8) under conditions found to be optimal for lipid remodeling in *T.brucei* microsomes (Morita et al., 2000). All labeled lipids are GPI intermediates and CP2, M4/1 and M4/2 are the most mature species, susceptible to be transferred onto proteins (Imhof et al., 2004). The result indicates that *gup1*p-deficient microsomes make a qualitatively normal spectrum of GPI intermediates and that the presence of C26:0-CoA, the presumed substrate of *gup1*p, does not change the mobility of the various lipids.

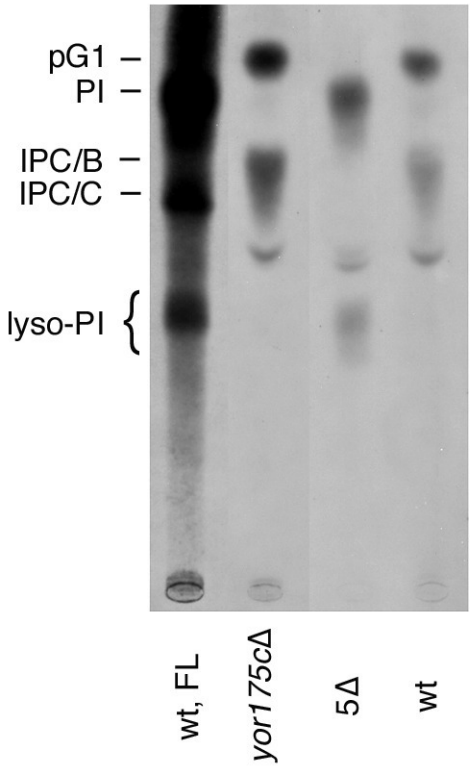
- Figure 3 - **Secretion of Gas1p is reduced when *GUP1* homologues are expressed in *gup1*· cells.**
  BY4742 (wt) or *gup1*· cells harboring different plasmids were grown on minimal SDaa medium. To analyze proteins secreted into the medium, the cultures were centrifuged, cells were washed with water and washes and medium were pooled. Proteins of the medium were precipitated with TCA, washed with acetone, and analyzed by SDS-PAGE and Western blotting. A cell extract (CE) of wt cells was added for control. Blots were decorated with antibodies against Gas1p or Sec61p, a membrane protein of the ER. One of two experiments giving the same result is shown. *gup1*· transfected with *tcGUP1* or *afGUP1* secrete less Gas1p into the medium than *gup1*· cells transfected with the empty vector. Sec1p is not detected in the medium, indicating that the proteins of the medium are not derived from lysed cells. Thus, it would

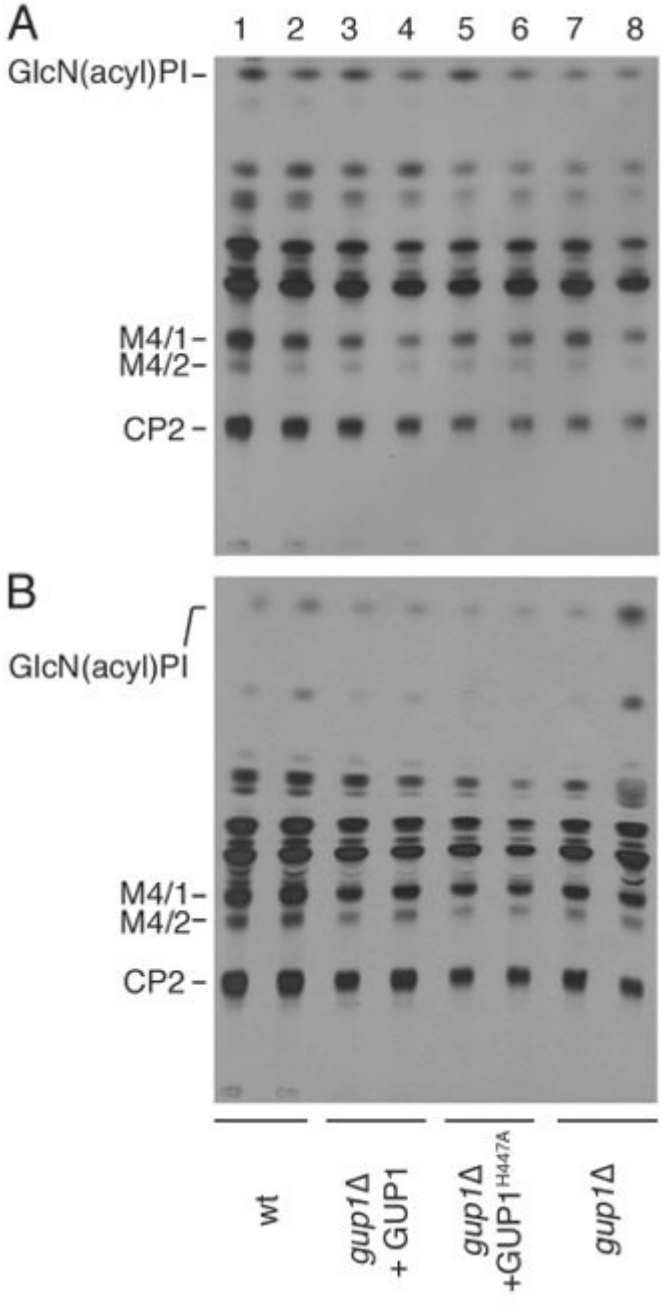
appear that the Gas1p secretion is partially corrected by expression of *tcGUP1* or *afGUP1*.

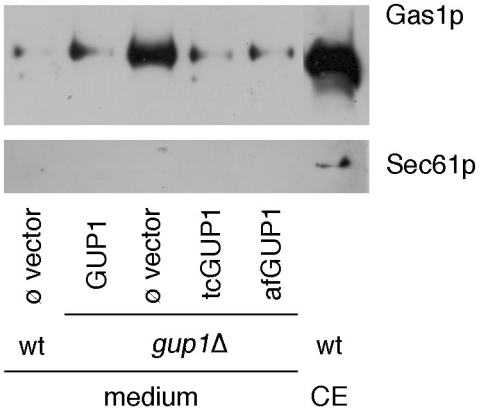
- Figure 4 - **Overexpression of *SLC1-1* does not rescue *gup1*·.**
  A, ten-fold dilutions of the *gup1*· and wt (BY4742) strains, containing either an empty vector (ø) or a single copy plasmid with *SLC1-1* under the control of a *GAL1* promoter, were spotted on SGaa plates containing zero, 50 or 100 •g/ml calcofluor white (CFW). The plates were incubated at 30°C for 3 days. **B,** wt (BY4742) or *gup1*· cells overexpressing *SLC1-1* were labeled at 30°C with [^{3}H]inositol, lipids of the GPI anchors were extracted and analyzed by TLC/fluorography using solvent 2. The first lane represents the free lipids of a wt strain (wt, FL).

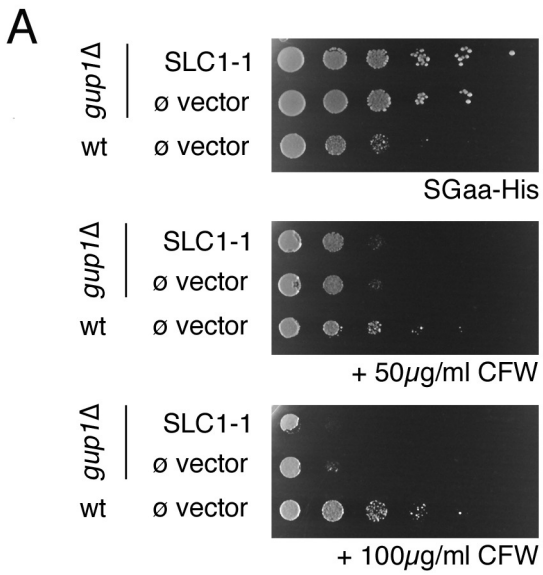
- Figure 5 - **Overexpression of *GUP1* does not rescue thermosensitivity of *lcb1-100*.**
  BY4742 wt strain and *lcb1-100* cells were transformed either with a plasmid containing *GUP1* under the control of the *GAL1* promoter or the empty vector (ø). Ten-fold dilutions of these strains were spotted on plates containing either glucose (SDaa) or galactose (SGaa) supplemented with 0 or 25 •M PHS. Plates were incubated at 24 or 37° C for 8 days.

- Figure 6 - **Secretion of CPY in *gup1*· strain.**
  A and **B,** 10-fold dilutions of the indicated cells were spotted on YPD medium and incubated for 24 hours at either 24°C (panel A) or 30°C (panel B). Then, a nitrocellulose membrane was placed on top of the cells and plates were further incubated for 16 hours at the same temperature. The nitrocellulose was washed, saturated with proteins and probed with anti-CPY antibody to reveal secretion of CPY. *Vps4*· was used as a positive control, as it has a vacuolar targeting defect and is known to secrete CPY. Upper panels represent the blot (anti-CPY) and lower panels the Petri dishes.


pG1
PI
IPC/B
IPC/C
lyso-PI
wt, FL
yor175cΔ
5Δ
wt


A
1 2 3 4 5 6 7 8
GlcN(acyl)PI
M4/1
M4/2
CP2
B
GlcN(acyl)PI
M4/1
M4/2
CP2
wt
gup1Δ + GUP1
gup1Δ +GUP1H447A
gup1Δ


Gas1p
Sec61p
o vector
GUP1
o vector
tcGUP1
afGUP1
wt
gup1Δ
wt
medium
CE


A
gup1Δ
SLC1-1
ø vector
wt
ø vector
SGaa-His
gup1Δ
SLC1-1
ø vector
wt
ø vector
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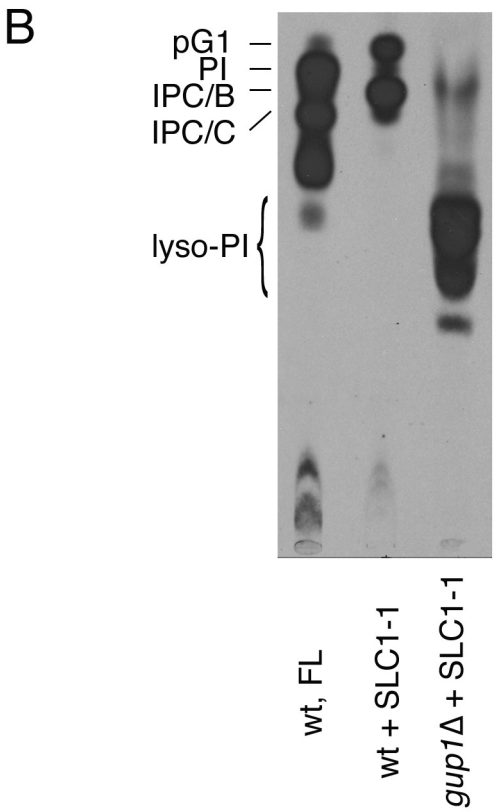
SLC1-1
ø vector
wt
ø vector
+ 100μg/ml CFW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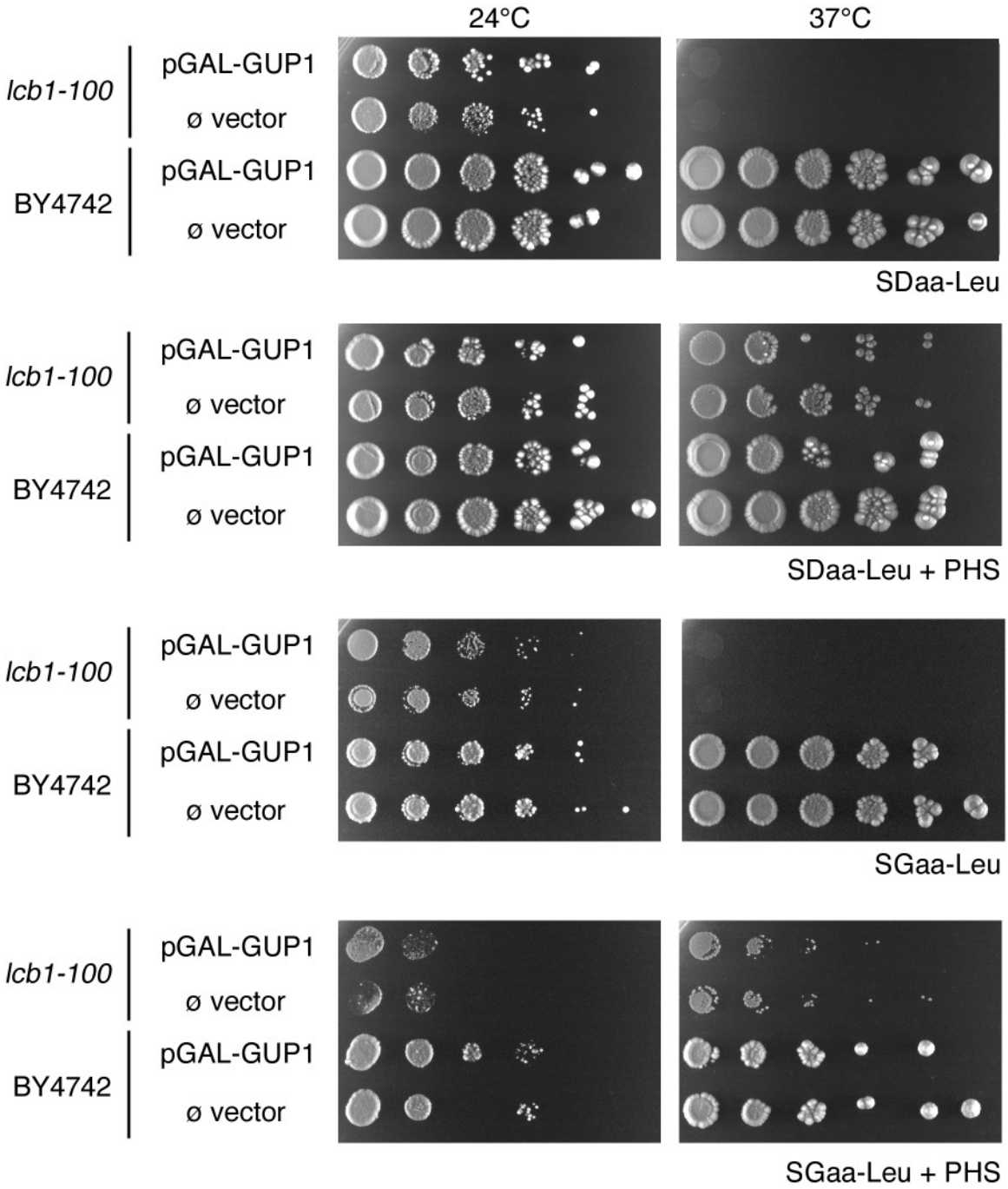
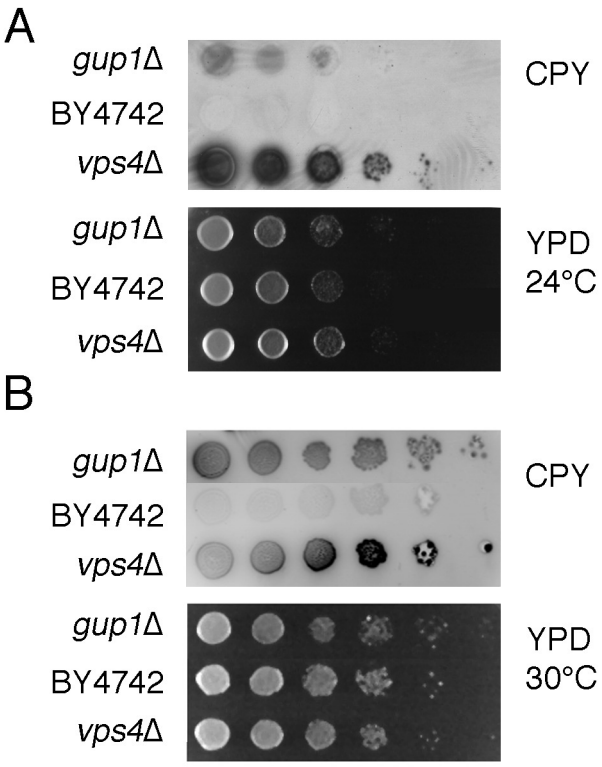
pG1
PI
IPC/B
IPC/C
lyso-PI
wt, FL
wt + SLC1-1
gup1Δ + SLC1-1


24°C
37°C
lcb1-100
pGAL-GUP1
ø vector
BY4742
pGAL-GUP1
ø vector
SDaa-Leu
lcb1-100
pGAL-GUP1
ø vector
BY4742
pGAL-GUP1
ø vector
SDaa-Leu + PHS
lcb1-100
pGAL-GUP1
ø vector
BY4742
pGAL-GUP1
ø vector
SGaa-Leu
lcb1-100
pGAL-GUP1
ø vector
BY4742
pGAL-GUP1
ø vector
SGaa-Leu + PHS


A
gup1Δ
BY4742
vps4Δ
CPY
gup1Δ
BY4742
vps4Δ
YPD
24°C
B
gup1Δ
BY4742
vps4Δ
CPY
gup1Δ
BY4742
vps4Δ
YPD
30°C